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Hemodynamic, Renal and Endocrine Effects of Atrial Natriuretic Peptide Infusion in Severe Heart Failure

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The cardiac release and total body and renal clearances and the hemodynamic, renal and endocrine effects of increasing doses of atrial natriuretic peptide were investigated in 12 patients with severe chronic congestive heart failure. Immunoreactive arterial plasma levels of atrial natriuretic peptide were 10-fold higher than normal and there was no correlation between aortic atrial natriuretic peptide and cardiac filling pressures. The heart released atrial natriuretic peptide into the coronary sinus. The kidney, though a major clearance site, accounted for only 33% of the total body clearance. Administration of 0.3 μg/kg per min atrial natriuretic peptide produced significant changes in heart rate (95 ± 4 to 85 ± 4 beats/min) and mean arterial (92 ± 8 to 77 ± 9 mm Hg), right atrial (13 ± 3 to 8 ± 2 mm Hg) and mean pulmonary artery occluded (27 ± 3 to 14 ± 3 mm Hg) pressures. Atrial natriuretic peptide increased cardiac index (2.25 ± 0.18 to 2.83 ± 0.3 liters/min per m^2) and stroke work index (21 ± 1.5 to 29 ± 3.4 g/m^2), whereas systemic vascular resistance (1,424 ± 139 to 1,033 ± 97 dynes·s·cm^{-5}) decreased.

Infusion of 0.1 μg/kg per min atrial natriuretic peptide increased urinary flow 128%, fractional excretion of sodium 133% and fractional excretion of potassium 35%. The filtration fraction increased from 29 ± 2 to 31 ± 4%. This represented a disproportionate rise in glomerular filtration rate over renal plasma flow. Plasma aldosterone and norepinephrine decreased whereas plasma renin activity remained unchanged. In association with these hemodynamic, excretory and endocrine changes, the urinary excretion of cyclic guanosine monophosphate doubled. Placebo had no effect. These results showed that, despite high circulating levels of atrial natriuretic peptide, administration of this hormone in heart failure is associated with potentially beneficial hemodynamic, renal and endocrine effects.

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Mammalian atrial cardiocytes contain secretorylike granules characteristic of endocrine tissue (1). Earlier studies (2, 3) have demonstrated that extracts prepared from rat atria contain factors that produce diuresis and natriuresis in vivo. Several structurally related peptides known collectively as atrial natriuretic factors have been isolated, purified and sequenced in animal (4) and human atria (5). The main peptide in these storage granules is the 126 amino acid, pro-atrial natriuretic peptide (gamma-hANP or $hANP_{1-126}$), but the principal circulating form in human plasma is probably the 28 amino acid, α-human atrial natriuretic peptide (alpha-hANP or $hANP_{99-126}$) (5,6). The physiologic and pharmacologic properties of these related peptides appear to reside in the C-terminal amino acid sequence. In addition to promoting diuresis and natriuresis, these peptides also have potent vasorelaxant (7,8) and endocrine effects such as inhibition of adrenocorticosteroid and renin secretion (9). Recent studies have examined the physiologic effects of atrial natriuretic peptide in humans. Infusions of low doses of this peptide in normal subjects have induced diuresis and natriuresis. After bolus infusions of atrial natriuretic peptide, some investigators (10) have reported renal but not endocrine effects. Others administering continuous infusions of doses ranging from 0.03 to 0.1 μg/kg per min have reported renal, vascular and endocrine effects (11).

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Table 1. Clinical Characteristics in 12 Patients

Patient	Age (yr) & Sex	Diagnosis	NYHA	Concomitant Medication	LVEF (%)
1	49M	CAD	III	Digoxin, warfarin, procainamide	15
2	55M	DCM	III	Digoxin, warfarin	20
3	52M	DCM	IV	Digoxin, warfarin, quinidine	10
4	54 F	DCM	III		10
5	33M	DCM	III	Digoxin, warfarin	10
6	55M	CAD	III	Digoxin, procainamide, tocainide	16
7	45M	CAD	III	Digoxin	10
8	53M	DCM	II		10
9	37M	DCM	II	Digoxin	10
10	57M	VHD	III	Warfarin, quinidine	10
11	40M	DCM	III	Tocainide	10
12	43M	DCM	II	Digoxin, Coumadin	10

CAD = coronary artery disease; DCM = idiopathic dilated cardiomyopathy; F = female; LVEF = left ventricular ejection fraction; M = male; NYHA = New York Heart Association functional class; VHD = valvular heart disease.

The role of atrial natriuretic peptide in heart failure is undetermined. Heart failure is associated with marked renal, endocrine and vascular abnormalities. Currently available treatments for heart failure accentuate many of these abnormalities by inducing activation of the adrenergic and renin-angiotensin-aldosterone systems. Because atrial natriuretic peptide appears to inhibit aldosterone and cortisol release in normal persons, its natriuretic, diuretic and vasorelaxant properties are potentially beneficial to patients with heart failure. Recently, immunoreactive levels of this peptide have been shown to be elevated in patients with heart failure (12,13). It is still unclear whether these elevated levels of immunoreactive peptide represent the 28 amino acid biologically active alpha-human atrial natriuretic peptide. We recently observed (14) that coronary sinus levels of immunoreactive peptide are higher than levels measured with a sensitive and specific biological assay. Bolus and continuous infusions of atrial natriuretic peptide in patients with heart failure, in doses equal to those shown to be effective in normal subjects, have failed to produce significant diuretic and natriuretic effects (11,15). The purpose of the present study was to determine the renal, hemodynamic and endocrine effects of increasing doses of a synthetic analog of atrial natriuretic peptide in patients with severe chronic heart failure. We also measured the release of this hormone by the heart and its renal and total body clearance.

Methods

Study patients (Table 1). Eleven men and one woman, aged 33 to 57 years, with class II to IV congestive heart failure (New York Heart Association criteria) gave informed consent after the experimental protocol was approved by the Hospital Ethics Committee. The study group consisted of patients referred to Stanford University Medical Center to be evaluated for heart transplantation. Eight patients had dilated idiopathic cardiomyopathy, three had coronary artery disease and one had aortic valve disease. Renal and hepatic function was adequate, as determined by normal serum creatinine and hepatic transaminase enzymes. Patients with a recent history of myocardial infarction, hypertension or urinary tract disease were excluded from the study. Three days before the study, all vasodilators, nonsteroid anti-inflammatory drugs, diuretic drugs and adrenergic and calcium channel blocking agents were discontinued (Fig. 1). Anticoagulants and antiarrhythmic agents and digitalis were not withheld.

The patients were admitted to the Clinical Research Center and given a caffeine-free 20 mEq sodium, 60 mEq potassium diet. On the morning of the 4th hospital day, the pulmonary and radial arteries were catheterized under fluoroscopic control and a catheter was inserted into the bladder. A priming and a sustaining infusion of para-aminohippurate and inulin was commenced. During pulmonary artery catheterization, blood samples for immunoreactive atrial natriuretic peptide were obtained in rapid sequence from the superior and inferior venae cavae, renal vein, coronary sinus, pulmonary artery and aorta. A peripheral vein catheter was also inserted for infusion of atrial natriuretic peptide.

Study design (Fig. 1). Baseline measurements were recorded in the coronary care unit after a 1 h equilibration period and after obtaining stable (<10% variation) right atrial, pulmonary artery occluded and radial artery pressures. Urinary flow rate and urinary sodium, potassium, inulin, para-aminohippurate, creatinine and cyclic guanosine monophosphate (cGMP) levels were measured every 30 min. At the beginning of the baseline period, time −60 min, the bladder was emptied and the urine discarded. An arterial blood sample was drawn for hematocrit, serum sodium, potassium, creatinine, inulin and para-aminohippurate. Urine and blood were then collected every 30 min for

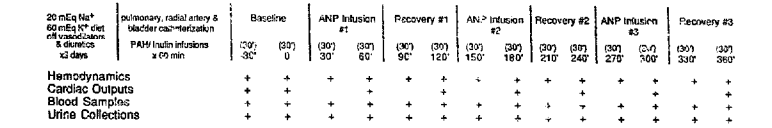

Figure 1. Schematic representation of the multiple dose atrial natriuretic peptide (ANP) infusion protocol design. Three patients received 0.03, 0.06 and 0.1 μg/kg per min, seven patients received 0.1, 0.2 and 0.3 μg/kg per min and two received infusions of 0.3, 0.45 and 0.6 μg/kg per min. Hemodynamic measurements included heart rate and right atrial, pulmonary artery occluded and systemic arterial pressures. Thermodilution cardiac output in triplicate was recorded hourly and systemic vascular resistance calculated accordingly. Blood and urine samples were obtained every 30 min for inulin, para-aminohippurate (PAH), cyclic guanosine monophosphate, sodium, creatinine and potassium clearances.

para-aminohippurate and inulin and hourly for hematocrit, creatinine and electrolyte determinations. Cardiac output was measured by thermodilution. Intracardiac pressures were recorded every 30 min, and cardiac output was measured in triplicate every 60 min.

Beginning at time 0 +120 and +240 min, the patients received 1 h infusions of alpha-human atrial natriuretic peptide$_{4\text{-}28}$ (alpha-hANP$_{4\text{-}28}$; Anaratide, Wyeth Laboratories). This agent is a 25 amino acid peptide that has similar biologic activity and the same amino acid sequence as the 28 amino acid alpha-human atrial natriuretic peptide, except that alpha-human atrial natriuretic peptide$_{4\text{-}28}$ lacks three amino acids from the amino terminus. A 1 h recovery period followed each 1 h infusion; hence, there were one baseline and three infusion and recovery periods. To identify a safe and effective dosage, the patients were sequentially assigned to a low (0.03, 0.06, 0.1 μg/kg per min), medium (0.1, 0.2, 0.3 μg/kg per min) or high (0.3, 0.45, 0.6 μg/kg per min) infusion regimen. Three patients received 0.03, 0.06 and 0.1 μg/kg per min, seven patients received 0.1, 0.2 and 0.3 μg/kg per min and two received 0.3, 0.45 and 0.6 μg/kg per min. Before the next incremental dose, blood was drawn for atrial natriuretic peptide determinations. The volume of urine excreted over the prior hour was replaced intravenously during the subsequent hour with 5% dextrose in 0.2% saline solution. At the end of the third recovery period, all catheters were removed and the patients transferred to the hospital's Clinical Research Center.

Protocol for plasma renin activity, aldosterone and norepinephrine determinations. On the following 2 days, the patients received a 2 h infusion of atrial natriuretic peptide on 1 day and a similar volume of 100 mg/ml mannitol in 0.005 *N* acetic acid as diluent placebo on the other day. The sequence of placebo and atrial natriuretic peptide was reversed in every other patient. The purpose of this protocol was to study the effects of atrial natriuretic peptide on the levels of plasma renin activity, aldosterone and norepinephrine and to determine the volume of distribution and total body clearance of the hormone. The studies were done in the morning. The first morning void started a 2 h baseline period, during which the patients attempted to void every 30 min. Before the end of the baseline period, with the patient in the supine position for >30 min, blood samples from an internal jugular catheter were drawn for hematocrit, plasma aldosterone, renin activity, atrial natriuretic peptide and norepinephrine. Blood was also collected for serum sodium, potassium, chloride, creatinine and osmolality. Heart rate and cuff blood pressure were recorded every 5 min. Three patients received a 2 h infusion of 0.1 μg/kg per min, and seven received an infusion of 0.3 μg/kg per min of the peptide. Renal and hemodynamic data were not analyzed in two of these seven patients because of either symptomatic hypotension or violations of the protocol. One patient received an infusion of 0.6 μg/kg per min. Patients were asked to urinate every 30 min. Blood was collected for immunoreactive peptide at 30 min and at the end of the infusion, and also at 5 and 15 min into recovery. Blood for hematocrit, serum osmolality, creatinine, sodium, potassium and chloride determinations was drawn at 60 min and at the end of the 2 h infusion and recovery periods. At the end of the 2 h recovery period, any urine voided during the experimental and recovery periods in excess of that predicted by the baseline rate was replaced by intravenous infusion of 5% dextrose in 0.2% saline solution over 3 h.

Nine patients completed and tolerated all phases of the protocol. One patient was removed from the study after the hemodynamic protocol because of anemia. Another patient was withdrawn because of unstable angina before the 2 h infusion of atrial natriuretic peptide, and a third was withdrawn because of symptomatic hypotension during infusion of 0.45 μg/kg per min.

Analytic determinations. Mean pressures were calculated by electronic integration (Hewlett-Packard). Thermodilution cardiac output, cardiac index, systemic vascular resistance and stroke volume index were calculated according to standard analytic techniques (16). Stroke work index (g/m^2) was calculated as stroke volume index·(MAP − POP)·0.0136, in which MAP denotes mean arterial pressure and POP denotes mean pulmonary artery occluded pressure.

The urinary volumes were measured using graduated cylinders. Serum and urinary sodium, potassium, chloride (milliequivalents per liter) and creatinine (milligrams per deciliter) concentrations were determined by standard analytic techniques. Serum and urinary osmolality (milliosmoles per kilogram) were measured by freezing point depression. Serum and urinary inulin and para-aminohippurate were assayed by spectrophotometry as previously described (17). The renal extraction of para-aminohippurate was assumed to be 85% (18). Renal blood flow was calculated by dividing para-aminohippurate clearance by 1-hematocrit. Fractional excretion was calculated as the clearance of the substance in question divided by the glomerular filtration rate. Urinary cyclic GMP was measured by radioimmunoassay (19). Plasma renin activity was measured as the rate of immunoassayable angiotensin I generated in plasma incubated at pH 7.4 at 37°C (20). Plasma aldosterone was determined by radioimmunoassay as previously described (20).

Atrial natriuretic peptide was measured by radioimmunoassay. Blood was collected into chilled tubes containing ethylenediaminetetraacetic acid (4.5 mg) and aprotinin (100 KIU/ml) and then centrifuged immediately at 4°C. Three milliliters of plasma were passed through a Sep-Pak C_{18} cartridge and atrial natriuretic peptide was eluted with 70% acetonitrile in 0.1% trifluoroacetic acid. Mean recovery (70 ± 2%) was calculated by adding ^{125}I-atrial natriuretic peptide to the sample and known amounts of synthetic atrial natriuretic peptide to pooled human plasma. After evaporation, the samples were resuspended in phosphate-buffered solution and assayed in duplicate. Synthetic human atrial natriuretic peptide (Peninsula Laboratories) was used to prepare standards in plasma and buffer. ^{125}I-atrial natriuretic peptide was obtained from Amersham. Immunoreactive atrial natriuretic peptide was measured with rabbit C-terminal specific anti-alpha-human atrial natriuretic peptide serum obtained from Peninsula Laboratories. This antibody exhibited 100% cross reactivity with alpha-human atrial natriuretic peptide$_{4-28}$ and 40% to gamma-human atrial natriuretic peptide$_{1-126}$. A 100 μl aliquot of antiserum was incubated with 100 μl of standard or sample for 24 h, and the labeled atrial natriuretic peptide was then added for an additional 16 to 20 h and incubated at 4°C. Free and bound fractions were separated by precipitation with goat anti-rabbit immunoglobulin serum after a 2 h incubation at room temperature. The sensitivity of this assay was 1.2 fm/tube. Total binding was 35%, and the 50% displacement point on a standard curve was 17 p*M*. Interassay and intraassay variability was <10%.

Total body clearance was calculated as the product of volume of distribution and elimination rate constant. The elimination rate constant was calculated as the slope of the natural logarithm of the plasma concentration versus time, and is equal to 0.693/elimination plasma half-life. Volume of distribution was calculated by dividing the continuous intravenous dose divided by the steady plasma concentration of the peptide and the elimination rate constant. The elimination half-life was calculated by dividing the natural logarithm of 2 by the elimination rate constant. There was no appreciable absorption of atrial natriuretic peptide by the infusion tubing. Plasma and infusate atrial natriuretic peptide were measured by radioimmunoassay.

Statistical analysis. Multiple regression analysis and analysis of variance for repeated measures, with subsequent Fisher and Dunnett *t* tests, were used to determine the significance of the changes observed. Paired and unpaired *t* tests were used to determine the significance of single comparisons. All values are expressed as mean ± SEM. Changes were considered significant at $p \leq 0.05$.

Results

Baseline cardiac filling pressures in the 12 patients were elevated. Mean right atrial pressure was 11 ± 2 and mean pulmonary artery occluded pressure was 26 ± 3 mm Hg. Despite normal serum creatinine, the glomerular filtration rate of 66 ± 6 ml/min per 1.73 m^2 and renal blood flow of 412 ± 36 ml/min per 1.73 m^2 were reduced to 50% of normal values (18).

Plasma atrial natriuretic peptide concentrations (Fig. 2). Plasma levels of atrial natriuretic peptide were determined from the superior and inferior venae cavae, pulmonary artery, renal vein and aorta in 8 of the 12 patients. The coronary sinus was sampled in only seven patients. The mean aortic plasma immunoreactive level of 242 ± 48 p*M* was significantly higher than the reported concentration in normal individuals (21) (Fig. 2a). Aortic and pulmonary artery natriuretic peptide concentrations were significantly higher than those in the renal vein and inferior vena cava. The concentration was highest in the coronary sinus. The kidney extracted 82 ± 2% of the renal artery atrial natriuretic peptide, and the estimated renal clearance was 189 ± 29 ml/min per 1.73 m^2. In this group of very sick patients, there was no correlation between arterial atrial natriuretic peptide concentration and right atrial or pulmonary artery occluded pressures (Fig. 2B and 2C).

Hemodynamic effects of atrial natriuretic peptide (Fig. 3). Three patients received 0.03, 0.06 and 0.1 μg/kg per min of atrial natriuretic peptide for 1 h. Seven patients had an infusion of 0.1, 0.2 and 0.3 μg/kg per min. By repeated analysis of variance and multiple regression analysis, the administration of atrial natriuretic peptide led to significant changes in heart rate ($r = 0.88$, $p \leq 0.0004$), right atrial pressure ($r = 0.95$, $p \leq 0.0001$), pulmonary artery occluded pressure ($r = 0.91$, $p \leq 0.0001$), cardiac index ($r = 0.84$, $p \leq 0.0001$) and systemic vascular resistance ($r = 0.78$, $p \leq 0.0001$). When baseline values are compared with maximal response, heart rate decreased from 95 ± 4 to 85 ± 4 beats/min ($p \leq 0.05$), mean arterial pressure from 92 ± 8 to 77 ± 9 mm Hg ($p \leq 0.05$), right atrial pressure from 13 ± 3 to 8 ±

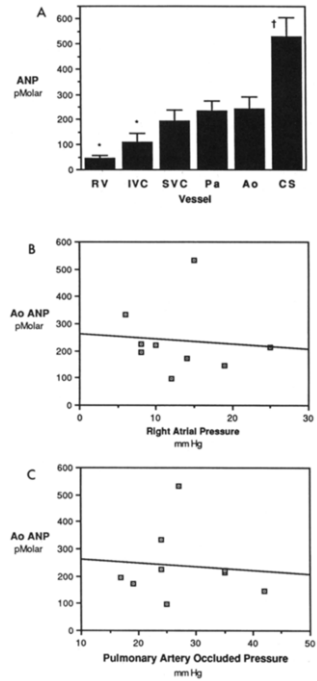

Figure 2. Plasma levels of atrial natriuretic peptide (ANP) (A) and the relation between right atrial (B) and pulmonary artery occluded (C) pressures with arterial plasma concentrations. Blood for immunoreactive atrial natriuretic peptide (mean ± SEM) from the renal vein (RV), inferior (IVC) and superior (SVC) venae cavae, pulmonary artery (Pa), aorta (Ao) and coronary sinus (CS) was obtained in rapid sequence in seven patients. The relation between cardiac filling pressures and arterial plasma concentrations (Ao ANP) was determined in 12 patients. *Denotes significant ($p \leq 0.05$) step-down between aortic and renal vein and inferior vena cava concentrations; † denotes significantly ($p \leq 0.05$) higher than superior and inferior venae cavae, pulmonary artery and aortic levels.

2 mm Hg ($p \leq 0.05$) and pulmonary artery occluded pressure from 27 ± 3 to 14 ± 3 mm Hg ($p \leq 0.05$). Cardiac index increased from 2.25 ± 0.18 to 2.83 ± 0.3 liters/min per m^2 ($p \leq 0.05$), whereas systemic vascular resistance decreased from 1,424 ± 139 to 1,033 ± 97 dynes·s·cm^{-5} ($p \leq 0.05$). Stroke work index increased from 21 ± 1.5 to 29 ± 3.4 g/m^2 ($p \leq 0.05$). Hemodynamic values tended to return to baseline during recovery. Atrial natriuretic peptide lowered heart rate and pulmonary artery occluded and right atrial pressures in a reversible manner. In contrast, blood pressure progressively decreased and did not return to preinfusion values during the 1 h recovery periods. However, the small but significant decrease in blood pressure during the 2 h infusion of 0.1 and 0.3 μg/kg per min atrial natriuretic peptide had returned to preinfusion values by the end of the 2nd h of the recovery period (Table 2).

Renal responses to atrial natriuretic peptide (Fig. 4). Ten patients received 0.1 μg/kg per min of atrial natriuretic peptide. Three of these patients received the infusion after the administration of 0.03 and 0.06 μg/kg per min of the hormone. Before the initiation of each higher dose, the plasma atrial natriuretic peptide concentration had returned to baseline values. There were no consistent renal changes at the 0.03 and 0.06 μg/kg per min doses. The other seven patients received 0.1 μg/kg per min at the beginning of the study. The data for the 0.1 μg/kg per min dose in the two groups were combined because there were no measurable renal effects at the 0.03 and 0.06 doses or any difference in the renal response of the two groups at the 0.1 μg/kg per min dose. Baseline renal function was markedly abnormal, consistent with severe heart failure. The administration of atrial natriuretic peptide led to significant changes in urinary flow rate and fractional excretion of sodium (FeNa) and potassium (FeK). In comparison with baseline values, urinary flow rate increased by 128% from 1.22 ± 0.25 to 2.79 ± 0.8 ml/min ($p \leq 0.05$), FeNa by 133% from 0.22 ± 0.11 to 0.52 ± 0.16% ($p \leq 0.05$) and FeK by 35% from 18.9 ± 2.4 to 25.6 ± 4.8% ($p \leq 0.05$).

During drug administration the glomerular filtration rate increased from 68 ± 6 to 81 ± 6 ml/min per 1.73 m^2 ($p \leq 0.10$), whereas the renal blood flow increased from 363 ± 19 to 440 ± 44 during the first 30 min and 395 ± 46 ml/min per 1.73 m^2 during the second 30 min period of infusion. These changes in renal blood flow were not statistically significant; however, the small increase in filtration fraction indicates a disproportionate elevation in glomerular filtration relative to renal plasma flow. The increment in filtration fraction was not associated with an elevation in renal vascular resistance. In association with these hemodynamic and excretory changes, the urinary excretion of cyclic GMP increased by 100% from 1,027 ± 315 to 2,091 ± 612 pmol/min. The renal excretion of cyclic GMP peaked during the first 30 min of recovery.

Table 2 shows the hemodynamic and renal effects of 2 h infusions of atrial natriuretic peptide and placebo in five patients. The administration of 0.3 μg/kg per min of atrial natriuretic peptide led to a small and reversible decrease in

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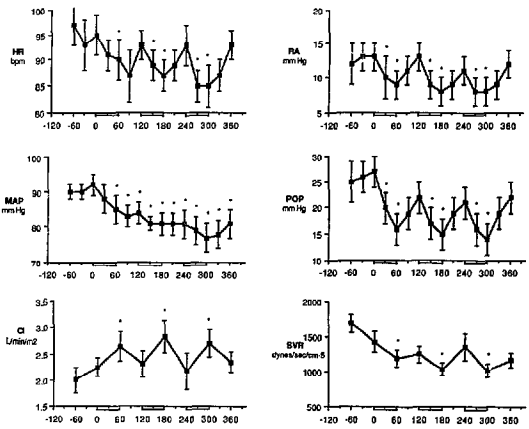

Figure 3. Heart rate (HR), mean arterial pressure (MAP), cardiac index (CI), right atrial (RA) and pulmonary occluded (POP) pressures and systemic vascular resistance (SVR) in the seven patients who received increasing doses of atrial natriuretic peptide (**bars on abscissa**). The patients received infusions of 0.1 (time 0 to 60), 0.2 (time 120 to 180) and 0.3 μg/kg per min (time 240 to 300 min) atrial natriuretic peptide. *Denotes significantly different ($p \leq 0.05$) from baseline values (mean ± SEM).

heart rate and mean arterial pressure. Similar to changes noted with 1 h infusions of atrial natriuretic peptide, there was a significant increment in urinary flow rate, urinary sodium excretion and fractional excretion of sodium. There was also an increase in urinary potassium excretion and its fractional excretion. Urinary osmolality dropped from 825 ± 92 to 573 ± 128 mOsmol/kg ($p \leq 0.05$). The baseline glomerular filtration rate in these five patients, as estimated by the creatinine clearance, was similar to the inulin clearances determined during the multiple dose protocol. Creatinine clearance during the infusion of 0.3 μg/kg per min of atrial natriuretic peptide increased from 69 ± 10 to 102 ± 28 ml/min, but this difference was not statistically significant. The infusion of 0.1 μg/kg per min of atrial natriuretic peptide led to parallel changes in renal function; however, given the small number of patients, these changes were not statistically significant. Placebo had no effect on any of the variables studied.

Endocrine effects and pharmacokinetics of atrial natriuretic peptide. Table 2 presents the endocrine changes observed during the 2 h infusions of various doses of atrial natriuretic peptide. As expected in severe heart failure, baseline plasma atrial natriuretic peptide, aldosterone, norepinephrine and plasma renin activity were markedly ele-

Table 2. Hemodynamic, Renal and Endocrine Effects of 2 Hour Infusions of Placebo and Atrial Natriuretic Peptide

Condition	irANP (pM)	HR (beats/min)	MAP (mm/Hg)	U·V (μl/min)	$U_{Na}V$ (μEq/min)	FeNa (%)	PRA (ng/ml per h)	PA (ng/dl)	NE (ng/l)
Baseline	308 ± 49	97 ± 4	89 ± 3	764 ± 239	3.5 ± 0.9	0.76 ± 0.36	30 ± 14	115 ± 39	902 ± 152
ANP	11891 ± 3327*	90 ± 3*	80 ± 3*	2934 ± 913*	29 ± 10*	2.45 ± 0.83*	53 ± 24	55 ± 20*	667 ± 114*
Recovery	—	97 ± 4	85 ± 2	1278 ± 305	14 ± 3.2	1.51 ± 0.35	—	—	—
Baseline	330 ± 50	91 ± 5	88 ± 3	757 ± 130	4.3 ± 1.3	0.41 ± 0.13	—	—	—
Placebo	320 ± 62	90 ± 4	88 ± 3	844 ± 220	6.8 ± 2.3	0.65 ± 0.27	—	—	—
Recovery		94 ± 4	88 ± 2	583 ± 150	3.5 ± 1.2	0.41 ± 0.15	—	—	—

*Denotes significantly different ($p \leq 0.05$) from values obtained during the preceding 2 h baseline period. After the multiple dose protocol, patients received a 2 h infusion of atrial natriuretic peptide on one day and a similar volume of 100 mg/ml mannitol in 0.005N acetic acid as the diluent placebo on the other day. The sequence of placebo and atrial natriuretic peptide was reversed in every other patient. Hemodynamic and renal excretory values (mean ± SE) are from five patients who received 0.3 μg/kg per min atrial natriuretic peptide. Endocrine data are from the nine patients who received various doses of the peptide. FeNa = fractional excretion of sodium; HR = heart rate; irANP = immunoreactive atrial natriuretic peptide; MAP = mean arterial pressure; NE = norepinephrine; PA = plasma aldosterone; PRA = plasma renin activity; $U_{Na}V$ = urinary sodium excretion; U·V = urinary flow rate.

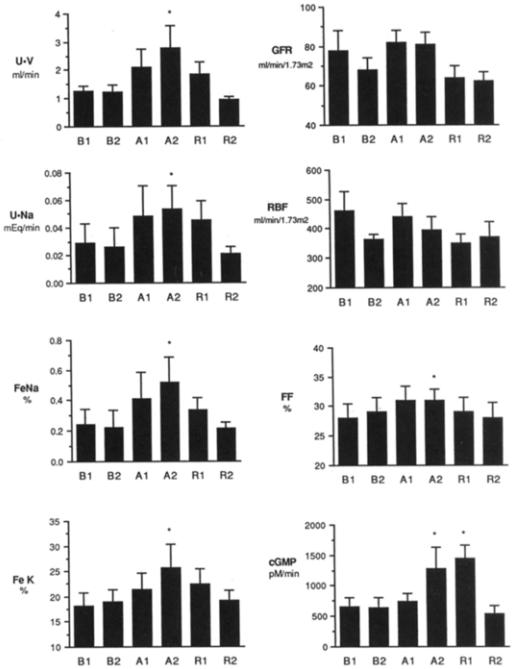

Figure 4. Renal excretory and hemodynamic responses to 0.1 μg/kg per min atrial natriuretic peptide infusion. Baseline (B1, B2), infusion (A1, A2) and recovery (R1, R2) periods were each 60 min. Urinary flow rate (U·V), sodium excretion (U·Na), fractional excretion of sodium (FeNa) and fractional excretion of potassium (FeK) were measured in the 10 patients who received 0.1 μg/kg per min atrial natriuretic peptide during the multiple dose protocol. Glomerular filtration rate (GFR), renal blood flow (RBF), filtration fraction (FF) and cyclic guanosine monophosphate (cGMP) were measured in only nine patients. *Denotes significantly different ($p \leq 0.05$) from baseline values (mean ± SEM).

vated. The 39-fold increment in plasma atrial natriuretic peptide at the end of the 2 h infusion was associated with a marked inhibition of plasma aldosterone and norepinephrine concentrations without a significant effect on plasma renin activity. All the doses of atrial natriuretic peptide had similar endocrine effects. Plasma aldosterone decreased 52% from 115 ± 39 to 55 ± 20 ng/100 ml ($p \leq 0.05$). Despite the small decrease in arterial blood pressure, plasma norepinephrine declined from 902 ± 152 to 667 ± 114 ng/liter ($p \leq 0.05$) without the expected increase in plasma renin activity.

Figure 5 illustrates the elimination half-life of exogenous atrial natriuretic peptide. The plasma half life was 4.3 ± 0.4 min. The calculated total body clearance of the peptide was 810 ± 133 ml/min. The elimination rate constant at these doses indicates that clearance of atrial natriuretic peptide probably follows zero order kinetics. The volume of distribution of the peptide was 5,155 ± 1,184 ml or 6 ± 1% of total body weight.

Discussion

Cardiorenal effects of atrial natriuretic peptide. In this study, we investigated the pharmacodynamic and pharmacokinetic properties of atrial natriuretic peptide in congestive heart failure. Infusion of synthetic atrial natriuretic peptide in patients with heart failure produced beneficial hemodynamic effects. These included arterial and venous vascular relaxation, with a concomitant decrease in pulmonary artery occluded and right atrial pressures and increased cardiac output and stroke work index. There was doubling of

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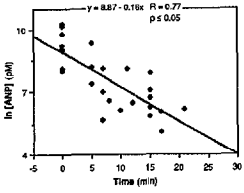

Figure 5. Plasma elimination half-life of exogenous atrial natriuretic peptide in nine patients. Blood was drawn for immunoreactive atrial natriuretic peptide (ln [ANP]) immediately, before, at 30 min and at 2 h into the infusion. After the infusion was discontinued (time 0), levels were obtained at 5 and at 15 min.

urinary flow rate, associated with natriuresis, and a slight increase in filtration fraction. Despite the decrease in arterial pressure and increased natriuresis, plasma aldosterone and norepinephrine levels declined, but plasma renin activity remained unchanged. Atrial natriuretic peptide was released into the coronary sinus and cleared by the peripheral tissues. The kidney was a major clearance site, extracting 82% of the arterial atrial natriuretic peptide. Renal clearance of endogenous atrial natriuretic peptide was determined 1 or 2 days before the exogenous clearance of atrial natriuretic peptide. If it is assumed that renal clearance was relatively stable, the kidney accounted for 33% of the total elimination of this hormone. The strikingly elevated arterial levels of atrial natriuretic peptide are probably due to enhanced production because the 4 min elimination half-life of the hormone in patients with heart failure does not appear to be prolonged when compared with values in healthy individuals (22). Atrial natriuretic peptide is also elevated in renal failure (23). Because the extrarenal tissues account for the principal disposal of this peptide (67%), the elevated immunoreactive levels present in chronic renal failure cannot be entirely explained by the decrease in renal clearance. Perhaps the elevated levels measured in renal failure represent, as in heart failure, enhanced release or impaired removal by extrarenal tissues, or both.

Plasma levels in heart failure. Atrial natriuretic peptide is believed to play an important role in volume homeostasis; therefore, it has been assumed that atrial pressure is an important determinant of atrial natriuretic peptide release. Some investigators (12,24) have shown a linear correlation between venous atrial natriuretic peptide and pulmonary artery occluded and right atrial pressures. We did not observe this relation. However, we compared cardiac filling pressures with arterial levels in this group of sick patients. Others (25) have also reported this lack of correlation. Perhaps it is not intraatrial pressure, but rather atrial wall tension and stretch that are the important determinants. Furthermore, as heart failure progresses, the volume homeostatic mechanisms are reset and vasoconstriction and fluid accumulation occur as if there were severe volume depletion (26,27). The role of atrial natriuretic hormone in this adaptation is not clear, but because infusion of atrial natriuretic peptide improved the hemodynamic, renal and endocrine derangements of heart failure, our data suggest that atrial secretion, and thereby arterial levels, may not be maintained at levels necessary to prevent fluid accumulation and vasoconstriction. This may be a primary atrial process such as would be expected from atrial cardiocyte secretory abnormalities or atrial cell loss, or both, due to atrial fibrosis and calicification as seen in chronic dilated heart failure (Billingham M, personal communication). Alternatively, atrial secretion may be inhibited by neurohumoral mechanisms activated in heart failure. Furthermore, the elevated level of immunoreacative atrial natriuretic peptide in heart disease may not completely correspond to biologically active peptide.

Arterial and venous vasodilation. Atrial natriuretic peptide relaxes precontracted arterial muscle strips in vitro, a process mediated by cyclic GMP (7,8). Furthermore, infusions of peptide into brachial artery in humans have increased forearm blood flow threefold by relaxing resistance vessels (28). Infusions of atrial natriuretic peptide into healthy volunteers (11) and animals (29,30) have lowered cardiac filling and arterial blood pressures without increasing cardiac output. The hypotensive effects of these peptides have been attributed to a decrease in cardiac index secondary to diuresis and venodilation (29). However, our data indicate that atrial natriuretic peptide reduced pulmonary artery occluded and right atrial pressure by increasing cardiac output and decreasing arterial and venous resistance. Therefore, the different hemodynamic effects of atrial natriuretic peptide observed in healthy subjects and patients with heart failure probably depend on the preexisting level of vascular tone.

In the present study, cardiac output increased and mean arterial blood pressure decreased, suggesting that atrial natriuretic peptide decreases peripheral resistance by exerting a direct vasorelaxant effect on the arterial circulation. The increase in cardiac output was due to an enhanced stroke work index, probably secondary to decreased impedance to flow and a decrease in heart rate. Alternatively, because we did not measure left ventricular volume, we have not excluded an effect of this peptide on left ventricular diastolic compliance or a direct inotropic effect. Furthermore, the increased cardiac output could be solely secondary to a more favorable position in the Starling curve as a result of decreased venous return. This possibility is unlikely because infusion of atrial natriuretic peptide was associated with a small decrease in arterial pressure.

In heart failure, atrial natriuretic peptide is apparently a

balanced vasodilator. Unlike atrial natriuretic peptide, venous vasodilators such as nitroglycerin and isosorbide dinitrate lower filling pressures by primarily reducing venous return and not by increasing cardiac output (31). Pure arterial vasodilators, such as nifedipine (32) and hydralazine (31), rarely lower cardiac filling pressures because the increased forward flow is compensated by enhanced venous return. Atrial natriuretic peptide relaxed the venous and arterial circulation, lowered heart rate and cardiac filling pressures and elevated cardiac index in a reversible manner. However, there was a progressive decrease in mean arterial blood pressure due to a slightly more prolonged effect on the systemic vascular resistance. The sustained arterial relaxant effects of atrial natriuretic peptide in humans have also been described by Bolli (28) and Crozier (15) and their coworkers.

Blunted renal response. Continuous or bolus infusions of atrial natriuretic peptide in normal subjects have produced significant renal excretory changes (10,11,33). Recent studies (11,15) involving small groups of patients with heart failure receiving either bolus or continuous infusions of low doses have failed to show any renal effects. In this study, we observed a doubling of urinary flow and sodium excretion. The reason for this discrepancy is not immediately obvious. Our patients were severely ill heart transplant candidates. Because congestive heart failure is a heterogenous syndrome, we speculate that the difference in the renal responses among studies could be attributable to interpatient variability in the different patient populations. Furthermore, the hemodynamic, renal and endocrine effects of diuretics or vasodilators in heart failure are not always beneficial because the baseline status of the patient is of importance in the pharmacodynamic effects of cardiac drugs. Alternatively, this difference could have been due to differences in bioavailability of the peptide.

It is unclear whether the renal effects of atrial natriuretic peptide observed in healthy animals and humans are due to direct tubular effects or represent changes in glomerular filtration rate. Maack et al. (30) reported in dogs an increase in glomerular filtration rate in response to atrial natriuretic peptide, but others have not found an effect in healthy subjects or patients with heart failure (10,11,15,33). In our study, the increases in urinary flow rate and sodium excretion elicited by both 0.1 and 0.3 μg/kg per min atrial natriuretic peptide were associated with an increase in filtration fraction. This was the result of a tendency for glomerular filtration to increase more than renal plasma flow. Interestingly, the small increase in filtration fraction was not associated with an increase in renal vascular resistance. There was an inverse correlation between urinary flow and sodium with renal vascular resistance (Fig. 6). This tendency toward lower renal vascular resistance suggests that atrial natriuretic peptide preferentially relaxed the afferent arterioles, an effect qualitatively different from that produced by infusion of norepinephrine and angiotensin II, which increase filtration fraction by preferentially contracting the efferent arterioles (34). In this respect, the renal hemodynamic effects of atrial natriuretic peptide appear to be unique.

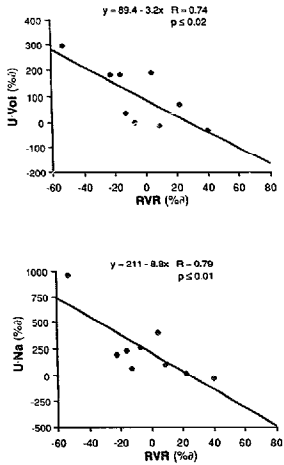

Figure 6. Relation between percent change from baseline in renal vascular resistance (RVR) and percent change in urinary flow rate (U·V) and urinary sodium excretion (U·Na) in the nine patients who received 0.1 μg/kg per min atrial natriuretic peptide and who had renal hemodynamic measurements.

Alternatively, we may speculate that the filtration fraction increased secondary to a change in the glomerular ultrafiltration coefficient (product of basement membrane permeability and glomerular filtration area). In our study, plasma oncotic pressure or hematocrit did not change (data not shown), but Cody (11) and Maack (30) and their coworkers noted that atrial natriuretic peptide in healthy subjects resulted in increased plasma oncotic pressure and hemoconcentration that were out of proportion to the diuresis observed. Furthermore, it has been reported (35) that atrial natriuretic peptide affects the permeability and hydraulic properties of capillaries. This peptide may affect the transcapillary efflux of endovascular fluid, either by affecting the capillary permeability properties or by increasing the intracapillary hydrostatic pressure, or both. Atrial natriuretic peptide is a very potent stimulator of cyclic GMP in cultured

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vascular endothelial cells (36,37). The physiologic significance of cyclic GMP stimulation in endothelial cells is not known, but we speculate that it may be responsible for changes in the permeability properties of capillaries.

Endocrine effects. The vasodilating and renal effects of atrial natriuretic peptide were associated with significant endocrine changes. Plasma aldosterone and norepinephrine decreased substantially whereas plasma renin activity remained unchanged. These endocrine effects may be either primary or secondary to the hemodynamic improvement. The decrease in plasma norepinephrine could be secondary to the increased cardiac index and decreased filling pressures. Alternatively, atrial natriuretic peptide may have a direct sympatholytic effect that contributed to the decline in systemic vascular resistance (38). Our data cannot answer this question, but similar hemodynamic changes induced by sodium nitroprusside in heart failure have not lowered plasma norepinephrine levels (32,39).

Plasma aldosterone levels were decreased 52% by atrial natriuretic peptide. The inhibition of plasma aldosterone was probably due to a direct inhibitory effect on the adrenal gland. Infusions into humans (11) and animals (30) and studies with isolated adrenal glands and zona glomerulasa cells (9,40,41) have shown a potent inhibitory effect on aldosterone and cortisol secretion. After the 2 h infusion of atrial natriuretic peptide, plasma aldosterone concentrations decreased from 115 ± 39 to 55 ± 20 ng/100 ml, a concentration that is still much greater than normal. The physiologic relevance of this observation is unknown. However, this brief reduction in plasma aldosterone is unlikely to be responsible for the observed renal changes because the cellular manifestations of aldosterone, being contingent on protein synthesis, require a greater time of onset.

Atrial natriuretic peptide lowered plasma aldosterone concentrations but had no effect on plasma renin activity. Most diuretics and vasodilators will stimulate the renin-angiotensin system. The absence of change in plasma renin activity despite the decrease in blood pressure and increased sodium excretion suggests that the peptide was exerting an inhibitory effect on the juxtaglomerular apparatus. The effect of atrial natriuretic peptide on plasma renin activity is controversial. Several investigators have reported decreases in plasma renin activity in humans (11) and in animals (30) whereas others have not (10,33,42). Atlas et al. (43) suggested that the effects of atrial natriuretic peptide on renin release are secondary to renal hemodynamic changes. However, the peptide has been shown to directly inhibit plasma renin activity release from rat renal cortex (44). Many of our patients were receiving angiotensin-converting enzyme inhibitors and diuretics up to 3 days before the study. These drugs are potent stimulators of renin release and they may have long-acting effects on basal renin production. Therefore, the mechanisms of action of atrial natriuretic peptide on plasma renin activity in heart failure remain to be determined.

Cyclic GMP has been proposed as the intracellular mediator of some atrial natriuretic peptide actions (7,38,45,46). Atrial natriuretic peptide stimulates cyclic GMP in a dose-dependent manner in the adrenal gland (47) and glomerulus and renal collecting ducts (45,48,49). Furthermore, cyclic GMP accumulation and cyclic GMP-dependent protein kinase activation to atrial natriuretic peptide precede vascular relaxation (7,8). In this study, urinary cyclic GMP, urinary flow and renal sodium excretion doubled. However, these renal effects, though similar in relative magnitude (twofold change), were in absolute terms significantly less than those produced in healthy individuals. Despite high circulating levels of endogenous atrial natriuretic peptide, basal renal excretion of cyclic GMP was not different from that observed in healthy euvolemic supine individuals (50). Furthermore, during infusion, the levels of atrial natriuretic peptide were 30 to 40 times higher than baseline values and 5,000 times higher than values measured in healthy water-immersed humans (51). However, this marked elevation in this peptide was only associated with a small natriuresis and increase in cyclic GMP excretion to levels that were lower than those observed in diuresing hypervolemic water-immersed healthy subjects. This observation suggests that the attenuated renal response to atrial natriuretic peptide observed in heart failure may be secondary to atrial natriuretic peptide receptor abnormalities. These may include down-regulation of the guanylate cyclase-coupled atrial natriuretic peptide receptor or abnormalities in guanylate cyclase activation after receptor binding. Alternatively, other neuroendocrine abnormalities may have inhibited atrial natriuretic peptide-induced natriuresis and diuresis by attenuating the activation of guanylate cyclase (52).

Conclusions. Infusion of several doses of atrial natriuretic peptide was associated with salutary hemodynamic, renal and endocrine effects. Atrial natriuretic peptide produced significant arterial and venous vasodilation and improved cardiac performance in association with a mild natriuresis and inhibition of plasma aldosterone and norepinephrine levels. These effects appear to be unique. The hemodynamic properties of atrial natriuretic peptide are very similar to those of sodium nitroprusside, except that atrial natriuretic peptide inhibits the neuroendocrine compensatory mechanisms that characterize vasodilator therapy. Most vasodilators stimulate sodium and fluid retention by decreasing glomerular filtration and activating the renin-angiotensin-aldosterone system. The renal and hemodynamic properties of atrial natriuretic peptide are singular in that they have no effect on sodium balance or they produce a moderate diuresis and natriuresis. This twofold improvement in renal excretory function fell short of being the major diuresis and natriuresis required of therapeutic diuretic agents. However,

the lack of stimulation of the renin-angiotensin-aldosterone system and secondary vasoconstriction, as seen with conventional diuretics (53), makes the further investigation of diuretic properties of atrial natriuretic peptide in heart failure enticing. Furthermore, the attenuated renal excretory and cyclic GMP effects of atrial natriuretic peptide in heart failure may be explained by abnormalities in the particulate guanylate cyclase-atrial natriuretic peptide-receptor system.

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